

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. RADX prevents MUS81-dependent DSBs (related to Figure 2). (A) Immunoblot to validate RADX siRNAs. (B) Representative images of U2OS cells transfected with non targeting siRNA (siNT) or siRNA targeting the RADX gene (siRADX) treated with 3mM HU for 4 hours and stained for BrdU and γ H2AX. (C) Representative images of the neutral comet assay performed in the absence of exogenous damage. (D) Cell cycle profiles of U2OS cells transfected with the indicated siRNAs were analyzed by flow cytometry using BrdU and propidium iodide staining. (E) Immunoblot to validate the RADX null cells obtained by CRISPR-CAS editing of the gene in U2OS and 293T cells (arrow points to RADX, *non-specific band). The non specific band is not observed in other blots because of better separation on lower percentage gels. (F) Immunoblot of RADX expression levels in U2OS and RADX Δ cells before and after complementation. (G) Immunoblot depicting knockdown of MUS81 and RADX. (H) DSBs in RADX wild-type and RADX Δ cells were measured by neutral comet assay 72 hours after transfection with the indicated siRNAs. P-values were calculated with a Kruskal-Wallis ANOVA and Dunn's multiple comparison post-test. The number of comet tails analyzed in each sample is indicated in parentheses. Experiment is representative of two replicates. Immunoblot to confirm knockdown of MUS81 is shown.

Figure S2. Multiple sequence alignment of RADX and RPA70 (related to Figure 3). Multiple sequence alignment of RPA70 and RADX from the indicated species was completed with CLUSTALW2.

Figure S3. RADX binds DNA through its RPA70-like OB-2 fold domain (related to Figure 3). (A) Coomassie stained SDS-PAGE gels of purified RADX from insect cells. Mass spectrometry indicated that the smaller molecular weight band is a RADX degradation product. (B)

Immunoblot depicting the complementation of RADX-proficient (WT) U2OS and RADX Δ cells with WT or OB-mutant RADX. Note that lanes 1,2,4, and 5 are also presented in Figure S1D.

(C) Sequence alignment of RPA70A and OB-2 of RADX. The cyan, blue and green dots indicate residues involved in hydrogen bonds, salt bridges and pi stacking interactions with DNA as observed in the crystal structure of RPA70AB in complex with DNA. (D) Ribbon representations of the RPA70-ssDNA complex (left, blue/cyan) extracted from the crystal structure of RPA70AB-dC8 (PDB code 1JMC) and a RADX OB-2 homology model (right, salmon). Residues selected for inclusion in the OBm mutant based on the model are highlighted.

Figure S4. RADX overexpression suppresses RAD51 foci formation and causes DNA damage (related to Figures 4 and 5). (A and B) Immunoblots after transfection with the indicated siRNAs in wild-type or RADX Δ U2OS cells. (C) U2OS cells transfected with non-targeting (NT) or RADX siRNA were labeled with 10 μ M BrdU for 22 hours prior to treatment with 3 mM HU for the indicated times. The cells were fixed and stained for BrdU using non-denaturing conditions to measure the amount of ssDNA. The mean+/-SEM from a representative experiment are depicted (n=107 per sample). (D) U2OS cells transfected with non-targeting (NT) or RADX siRNA were left untreated or treated with 3mM HU for 24 hours. The soluble and chromatin fractions were immunoblotted for the indicated proteins. (E) Mock or Flag-RADX overexpressing U2OS cells were labeled with 10 μ M EdU for thirty minutes prior to staining for EdU and RADX. The percentage of EdU positive cells is presented (mean+/-SEM, n=3). P-value derived from a Mann Whitney test. (F) Cell cycle profiles of mock and GFP-RADX overexpressing U2OS cells were analyzed by propidium iodide staining and flow cytometry. (G) Mock and GFP-RADX overexpressing U2OS cells were stained for γ H2AX. The mean+/-SEM from a representative experiment is presented (n=144). P-value was derived from a Mann Whitney test. All representative experiments were repeated at least twice.

Figure S5. RADX deletion causes excessive RAD51 activity at forks and restores fork protection without restoration of HR in BRCA2/RAD51-compromised cells (related to Figures. 5, 6 and 7). (A-D) Immunoblots depicting RADX, BRCA2, SMARCAL1 or ZRANB3 knockdown as indicated. (E) Summary of the median CldU/IdU ratio and number of fibers analyzed for the single-molecule nascent strand degradation assay depicted in Figure 6. (F) The percentage of GFP-positive DR-GFP-U2OS cells after transfection with the indicated siRNAs and I-SceI expression vector was measured by flow cytometry. The concentration indicates the amount of RAD51 siRNA used in each sample. The mean and SD from three experiments in which 25,000 cells were scored is depicted. (G) Mock and GFP-RADX overexpressing U2OS cells were irradiated (3 Gy) and then allowed to recover for the indicated times before staining for RAD51. The number of cells with >10 RAD51 foci after detergent extraction is presented (Mean+/-SEM from n=3). P value derived from a two-way ANOVA with Sidak's post test for multiple comparisons. (H) The percentage of GFP-positive DR-GFP-U2OS cells after transfection with the indicated vectors was measured by flow cytometry. The mean and SEM from three experiments in which 25,000 cells were scored is depicted. (I) Parental or RADX Δ U2OS cells transfected with the BRCA2 siRNAs were either untreated or treated with 2Gy. Surviving cell colonies were stained and scored. (mean+/-SD, n=3). The experiment was completed in triplicate.

Figure S6. RADX and RAD51 expression correlations with patient outcome (related to Figure 6). (A-D) Kaplan Meier plots of RADX and RAD51 mRNA expression and correlation with patient outcome in breast and lung cancers were derived from KM plotter (Szász et al. 2016). Settings for all graphs were as follows: auto select best cutoff, only JetSet best probe, remove redundant samples, and exclude biased arrays.

Figure S7. Model for RADX function (related to Figures 1-7). (A) In unstressed cells, RADX prevents inappropriate RAD51-dependent fork reversal. In RADX Δ cells, RAD51 accumulates at forks causing fork cleavage by the MUS81 endonuclease. (B) A balance of RAD51 activity is maintained at stalled replication forks by RADX and BRCA2. In BRCA2-mutant cells, there is insufficient RAD51 to prevent MRE11-dependent fork degradation. However, deletion of RADX restores fork protection and also yields decreased sensitivity to PARP inhibitors. See text for more details.

Figure S1 (related to Figure 2)

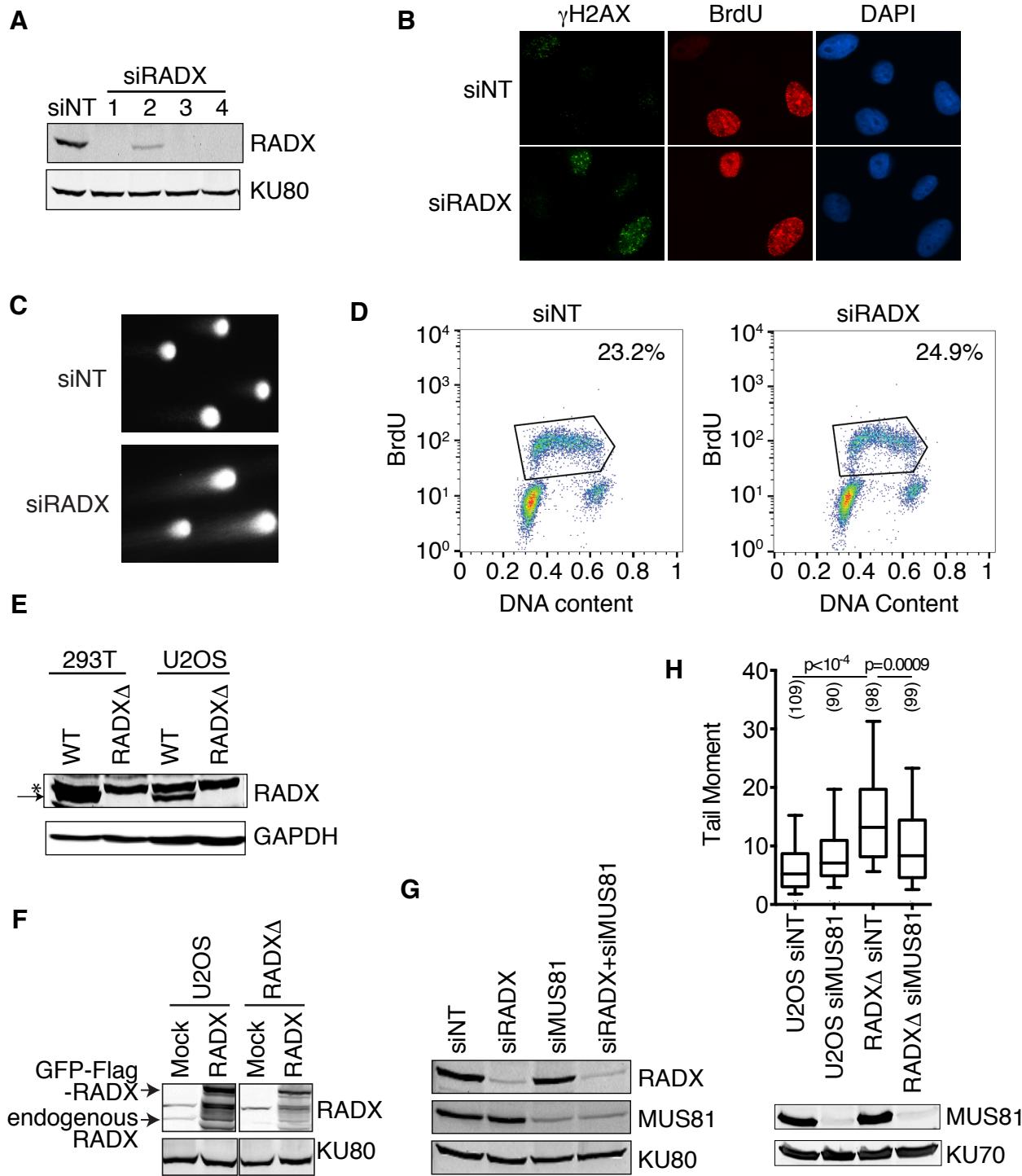


Figure S2 (related to Figure 3)

human_RPA1/1-616	1	MVG-Q - LSEG -	- A1 AAI MO - KGDTNKIP1 LQ -	- V1 N1 RPI TTGNSPRYY - RLLMS DGLNLT 53	
bovine_RPA1/1-616	1	MVG-H - LSEG -	- A1 AA1 MO - QGDTSIKPI LQ -	- V1 N1 RPI TTGNSPRYY - RLLMS DGLNLT 53	
canine_RPA1/1-616	1	MVG-H - LSEG -	- A1 AA1 MO - QGETSIKPI LQ -	- V1 N1 RPI TTGNSPRYY - RLLMS DGLNLT 53	
rat_RPA1/1-680	1	MO -	- QGDTSIKPVLO -	- V1 N1 RPI TTGNSPRYY - RLLMS DGLNLT 40	
mouse_RPA1/1-623	1	MVG-H - LSEG -	- A1 EVMIQ - QENTS1KPI LQ -	- V1 N1 RPI STGNRSRPRY - RLLMS DGLNLT 53	
frog_RPA1/1-609	1	MALPQ - LSEG -	- A1 SIA - ML - GGDSCKPTLQ -	- V1 N1 RPI TTGNGPYRY - RLLMS DGLNLT 53	
frog_RADX1-839	1	MHA - LLSAE FDEGGPVPSRLG -	- PLSES DTDGSLWTQGTRQRQVDRDCPSLTLV LQEPVPVLV LAVLRLY LGPPGRY -	- TFCYDVYI LWGDAGQD 88	
rat_RADX1/1-844	1	MSG-E - SDQOPQGPSPSHAGLYLEHRRDQAGVPGVV	- IRAGSQRHRWSV QTIVI QT1 TGSPRCVTLSEVPVSVLAVLQRY LLEDEPRDTPVKPKLYC YCDVTI 1 SDGVYYQE 106		
canine_RADX1/1-862	1	MSG-L - SGQPGQGPSPSHAGLDFPNPDRNRAVGPGGVV	- IRAGSQRHRWSV KQV1 LEQI TDSPLQWV TPSEEVPAVAVLQRY LLEDEPRDTPVKPKLYC YCDVTI 1 SDGVYYQE 106		
mouse_RADX1-850	1	MSG-E - SGQPGQGPSPSHAGLDPNPERNRNAVGPGGVV	- IRAGSQRHRWSV KQV1 LEQI TDSPRQCVCVTESEVPAVTVLAVLQRY LLEDEPRDTPVKPKLYC YCDVTI 1 SDGVYYQE 106		
human_RADX1-855	1	MSG-E - SGQPEAGPSHAGLDWPNPERNRNAVGPGGVV	- IRAGSQRHRWSV KQV1 LEQI MDSPRQCVCVTESEVPAVTVLAVLQRY LLEDEPRDTPVKPKLYC YCDVTI 1 SDGVYYQE 106		
bovine_RADX1-855	1	MSG-E - SGQPGQDPSQAGVQDMFPERNRGAAPVRGVYRVRVGA LGRSSW1 KVFEQV1 ISDPRRW TLPEVVPVAVALQRY LLEDEPRNPKPKPLYCFDVTI 1 SDGVYYQE 106			
human_RPA1/1-616	54	SFFMLATQLNPVVEEQI SSNCVCIK1 HRF -	- IVNTLKDGRVV1 LME LEV LKSAEAVGVK J GNPVPVYNE - GLGO - PQ -	- VAPPAPAASPAASSRPPQPNQNSG 150	
bovine_RPA1/1-616	54	SFFMLATQLNPVVEEI SSNC1 CQ1NRF -	- IVNTLKDGRVV1 LME LEV LKSAEAVGKJ GNPVPVYNE - GHGO - QP -	- VPPVPPSATSPP - TSRQPNQNSG 150	
canine_RPA1/1-616	54	SFFMLATQLNPVVEEEQI SSNC1 CQ1NRF -	- IVNTLKDGRVV1 LME LEV LKSAEAVGKJ GNPVPVYNE - GHGO - QP -	- VPPVPPSATSPP - TSRQPNQNSG 150	
rat_RPA1/1-680	41	SFFMLATQLNLA VEGGQLASNCVQCVHFR -	- IVNTLKDGRVV1 LMD LEV VVKSAEVEGV1 J GNPVPVYNE - GHQO - QQ -	- AVPSVPSVAANP - ANPKPQQNGSA 150	
mouse_RPA1/1-623	54	SFFMLATQLNLT VEGGQLASNCVQCVHFR -	- IVNTLKDGRVV1 LMD LEV VVKSAEVDGLK J GNPVPVYNE - GHQO - QQ -	- AVSAPASAATPP - ASKPQPNQNS LG 137	
frog_RPA1/1-609	54	SFFMLATQLNLS VDNN1 LATNC1 CQVSFR -	- IVNVLKDGRVV1 I VME LDVLVKSADLVMKG1 K PONPYND - GQP - QP -	- AAPAPASAPAPA - PSKLONNSA - 146	
frog_RADX1-839	89	VVMH - LHSDDNLVLYVN1 LRSGARV1 TSRCSYMSKEKS LDRGVL CVCEVELLS - GELTAL -	- - PNAQHLLPAAG - Q -	- LPLLG 160	
rat_RADX1/1-844	107	KCY - LDPSLNLVLYVON1 LVKG1 EMR1 SVRSVCLYNEKRG1 GOG1 LC1 DVKHCGEP LDV1 SV -	- - ETFRNRAHEE - KP -	- RPLRG 183	
canine_RADX1/1-862	107	KCY - LDPSLNLVLYVON1 LVKG1 EMR1 SVRSVCLYNEKRG1 GOG1 LC1 DVNHCGEALAE1 SL -	- - ETFRNRAHEE - KP -	- RPLRG 183	
mouse_RADX1-850	107	KCY - LDPSLNLVLYVON1 LVKG1 EMR1 SVRSVCLYNEKRG1 GOG1 LC1 DVNHCGESLDV1 SV -	- - ETFRNRAHEE - KP -	- RPLRG 183	
human_RADX1-855	107	KCY - LDPSLNLVLYVON1 LVKG1 EMR1 SVRSVCLYNEKRG1 GOG1 LC1 DVNHCGETSDS1 SL -	- - ETFRNRAHEE - KP -	- RPLRG 183	
bovine_RADX1-855	107	KCY - LDPSLNLVLYVON1 LVKG1 EMR1 FRVSC1 YNEKRG1 GOG1 LC1 DVNLVCGELLESVSL -	- - ETFRNRAHEE - VP -	- RPLRG 183	
human_RPA1/1-616	151	MGS TSKAYGASKTFGKAAGPSL SHSGTSQKVFPV1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 LEV 251		
bovine_RPA1/1-616	151	MAS TASKAFGASKTFGKAGGTQSNSGGTQAKVVF1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QADKF FFP1 DV 251		
canine_RPA1/1-616	151	MGTASKTYSASKTRGPKGPTLASSGGTQKVVF1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 DM 251		
rat_RPA1/1-680	138	VGS TAKAYGASKTFGKAAGPSL SHSGTSQKVFPV1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 238		
mouse_RPA1/1-623	160	MGS TAAYAKGASKTFGKAAGPSL SHSGTSQKVFPV1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242		
frog_RPA1/1-609	147	- - PPPSPMRTGTSKLFG -	- - GGSLLNTPQGGSQKVFPV1 I ASLPTPYQSKW -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242	
frog_RADX1-839	161	GRKHYL P LWNNEPD YGDI Y TDWTNGPK - LAEDQVS1 IDVSKVSS LORLVEQVTRRSLPPLV1 LVR1 MGS TSKAYGASKTFGKAAGPSL SHSGTSQKVFPV1 I ASLPTPYQSKW -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242		
rat_RADX1/1-844	147	- - GGSLLNTPQGGSQKVFPV1 I ASLPTPYQSKW -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242		
canine_RADX1/1-862	147	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242			
mouse_RADX1-850	147	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242			
human_RADX1-855	147	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242			
bovine_RADX1-855	147	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242			
human_RPA1/1-616	151	MGS TSKAYGASKTFGKAAGPSL SHSGTSQKVFPV1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 LEV 251		
bovine_RPA1/1-616	151	MAS TASKAFGASKTFGKAGGTQSNSGGTQAKVVF1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QADKF FFP1 DV 251		
canine_RPA1/1-616	151	MGTASKTYSASKTRGPKGPTLASSGGTQKVVF1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 DM 251		
rat_RPA1/1-680	138	VGS TAKAYGASKTFGKAAGPSL SHSGTSQKVFPV1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 238		
mouse_RPA1/1-623	160	MGS TAAYAKGASKTFGKAAGPSL SHSGTSQKVFPV1 I ASLPTPYQSKW -	- TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242		
frog_RPA1/1-609	147	- - PPPSPMRTGTSKLFG -	- - GGSLLNTPQGGSQKVFPV1 I ASLPTPYQSKW -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242	
frog_RADX1-839	161	GRKHYL P LWNNEPD YGDI Y TDWTNGPK - LAEDQVS1 IDVSKVSS LORLVEQVTRRSLPPLV1 LVR1 MGS TSKAYGASKTFGKAAGPSL SHSGTSQKVFPV1 I ASLPTPYQSKW -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242		
rat_RADX1/1-844	147	- - GGSLLNTPQGGSQKVFPV1 I ASLPTPYQSKW -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242		
canine_RADX1/1-862	147	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242			
mouse_RADX1-850	147	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242			
human_RADX1-855	147	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242			
bovine_RADX1-855	147	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLELVEDSEGE RATAFNE QVDFK FFP1 IEV 242			
human_RPA1/1-616	252	NK VYY FSKGTLK1 ANK - QFTAVKNDYEMT -	- - FNNE TSVMP CEDDHHLPTVQFDFTG1 DDLLENKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
bovine_RPA1/1-616	252	NK VYY FSKGTLK1 ANK - QFTAVKNDYEMT -	- - FNNE TSVMP CEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDVTF I VTKSNNRE - V 341		
canine_RPA1/1-616	252	NK VYY FSKGTLK1 ANK - QFTAVKNDYEMT -	- - FNNE TSVMP CEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEATK TVKSNNRE - V 341		
rat_RPA1/1-680	239	NK VYY FSKGTLK1 ANK - QFTAVKNDYEMT -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVKSNNRE - V 328		
mouse_RPA1/1-623	239	NK VYY FSKGALK1 ANK - QFSAVKNDEMT -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVKSNNRE - V 328		
frog_RPA1/1-609	239	NK VYY FSKGALK1 ANK - QFSAVKNDEMT -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVKSNNRE - V 328		
frog_RADX1-839	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QADKF FFP1 LEV 251	
rat_RADX1/1-844	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QADKF FFP1 DV 251	
canine_RADX1/1-862	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QVDFK FFP1 DM 251	
mouse_RADX1-850	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QVDFK FFP1 IEV 238	
human_RADX1-855	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QVDFK FFP1 IEV 242	
bovine_RADX1-855	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QVDFK FFP1 IEV 242	
human_RPA1/1-616	252	NK VYY FSKGTLK1 ANK - QFTAVKNDYEMT -	- - FNNE TSVMP CEDHHHLPTVQFDFTG1 DDLLENKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
bovine_RPA1/1-616	252	NK VYY FSKGTLK1 ANK - QFTAVKNDYEMT -	- - FNNE TSVMP CEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDVTF I VTKSNNRE - V 341		
canine_RPA1/1-616	252	NK VYY FSKGTLK1 ANK - QFTAVKNDYEMT -	- - FNNE TSVMP CEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEATK TVKSNNRE - V 341		
rat_RPA1/1-680	239	NK VYY FSKGALK1 ANK - QFSAVKNDEMT -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVKSNNRE - V 328		
mouse_RPA1/1-623	239	NK VYY FSKGALK1 ANK - QFSAVKNDEMT -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVKSNNRE - V 328		
frog_RPA1/1-609	239	NK VYY FSKGALK1 ANK - QFSAVKNDEMT -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVKSNNRE - V 328		
frog_RADX1-839	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QADKF FFP1 LEV 251	
rat_RADX1/1-844	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QADKF FFP1 DV 251	
canine_RADX1/1-862	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QVDFK FFP1 DM 251	
mouse_RADX1-850	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QVDFK FFP1 IEV 238	
human_RADX1-855	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QVDFK FFP1 IEV 242	
bovine_RADX1-855	239	NK VYY FSKGALK1 ANK - QFTAVKNDYEMT -	- - FNSESTV1 I PCDDSDADVPMQVF1 I PEQYQTY -	- - TICARV1 TNKSO1 RTWSNSRG - EGKLF1 SLEVDESEGE RATAFNE QVDFK FFP1 IEV 242	
human_RPA1/1-616	342	AKRN1 Y LMDF1 TSGKV - VTATLGED - ADKF -	- - DGSRQPV LA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
bovine_RPA1/1-616	342	SKRN1 Y LMDDMSGKV - VNATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
canine_RPA1/1-616	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
rat_RPA1/1-680	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
mouse_RPA1/1-623	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
frog_RPA1/1-609	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
frog_RADX1-839	371	GVI LLLQOYFSKKSYPLR1 I QDPDVPQDMK1 LSTME -	- - FNNE TSVMP CEDHHHLPTVQFDFTG1 DDLLENKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
rat_RADX1/1-844	293	GL1 LLLQDYSVKKSYPLR1 I QDPDVPQDMK1 LSTME -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
canine_RADX1/1-862	293	GL1 LLLQDYSVKKSYPLR1 I QDPDVPQDMK1 LSTME -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
mouse_RADX1-850	293	GL1 LLLQDYSVKKSYPLR1 I QDPDVPQDMK1 LSTME -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
human_RADX1-855	293	GL1 LLLQDYSVKKSYPLR1 I QDPDVPQDMK1 LSTME -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
bovine_RADX1-855	293	GL1 LLLQDYSVKKSYPLR1 I QDPDVPQDMK1 LSTME -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
human_RPA1/1-616	342	AKRN1 Y LMDF1 TSGKV - VTATLGED - ADKF -	- - DGSRQPV LA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
bovine_RPA1/1-616	342	SKRN1 Y LMDDMSGKV - VNATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
canine_RPA1/1-616	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
rat_RPA1/1-680	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
mouse_RPA1/1-623	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
frog_RPA1/1-609	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - DGSRQPVMA1 -	- - KGA RVSDFGGRSLSV - LSSST1 -- 399	
frog_RADX1-839	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - FNNE TSVMP CEDHHHLPTVQFDFTG1 DDLLENKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
rat_RADX1/1-844	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
canine_RADX1/1-862	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
mouse_RADX1-850	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
human_RADX1-855	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
bovine_RADX1-855	342	SKRN1 Y LMDDMSGKV - VTATLGED - ADKF -	- - FNNE TSVLP CLEDGHHLPTVQFDFTG1 GDLESKSKDSLVD1 I G1 CKSYEDATK TVRSNNRE - V 341		
human_RPA1/1-616	400	- - I ANDP1 PEAKYLRGW -	- - FDEAQGLDQ1 S LDKSGGSVHGG 437		
bovine_RPA1/1-616	400	- - I VNDP1 PEAKYLRGW -	- - FDEAQGLDQ1 S I SLDLRSGGGG 437		
canine_RPA1/1-616	400	- - I VNDP1 PEAKYLRGW -	- - FDEAQGLDQ1 S I SLDLRSGGGG 437		
rat_RPA1/1-680	387	- - I VNDP1 PEAKYLRGW/HYV FGDGGLKALLH - RQAHDP L MNM LESQRKOLELKCCVLT1 I PPGQGFNSCVLESSAES1 I SGLRCQQLMVGFDSE QGALDGV1 S I SLDLRSGGGG 437	- - FDEAQGLDQ1 S I SLDLRSGGGG 437		
mouse_RPA1/1-623	409	- - I VNDP1 PEAKYLRGW -	- - FDEAQGLDQ1 S I SLDLRSGGGG 437		
frog_RPA1/1-609	391	- - I MNDP1 PEAKFLRRAW -	- - FDEAQGLDQ1 S I SLDLRSGGGG 437		
frog_RADX1-839	486	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - CQHAVL -	- - FDESEQVVEGTS1 I SESRGGTTG 428	L - 562
rat_RADX1/1-844	505	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - FVNAS -	P - 581
canine_RADX1/1-862	505	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - FVNAS -	R - 582
mouse_RADX1-850	506	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - FVNAS -	R - 582
human_RADX1-855	506	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - FVNAS -	R - 582
bovine_RADX1-855	506	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - FVNAS -	R - 582
human_RPA1/1-616	505	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - TLRNAK -	R - 581
bovine_RPA1/1-616	505	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - TLRNAK -	R - 581
canine_RPA1/1-616	505	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - TLRNAK -	R - 581
rat_RPA1/1-680	505	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - TLRNAK -	R - 581
mouse_RPA1/1-623	505	- - I YQP1 I TPK1 PFKYCK - DNTAGE1 I LKLFSELKET1 I SQLHYERKRV1 QGT1 I A1QG1 I TAK1 Y PIYVHNSAKSAPASE -	- - TLOQAS -	- - TLRNAK -	

Figure S3 (related to Figure 3)

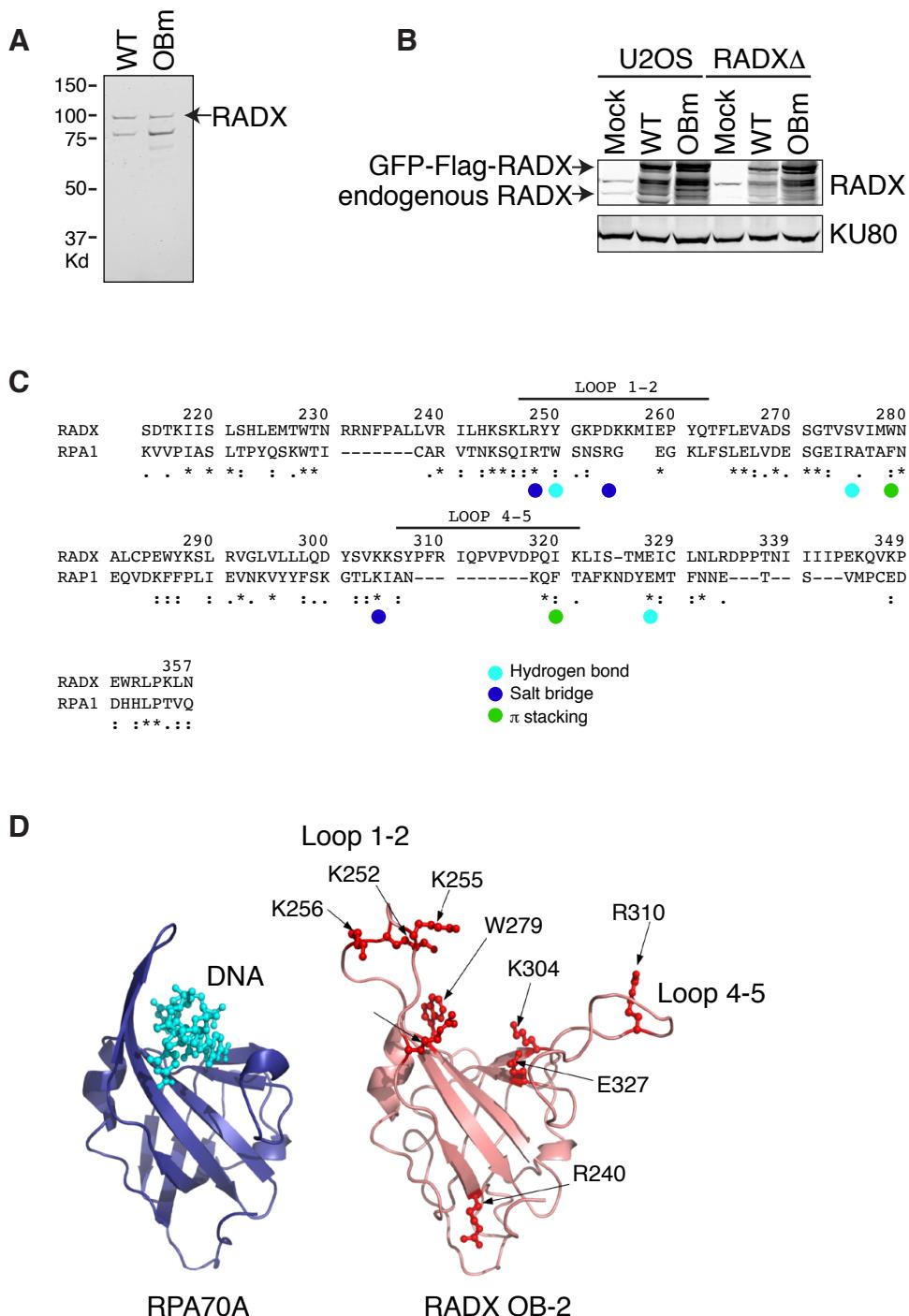


Figure S4 (related to Figures 4 and 5)

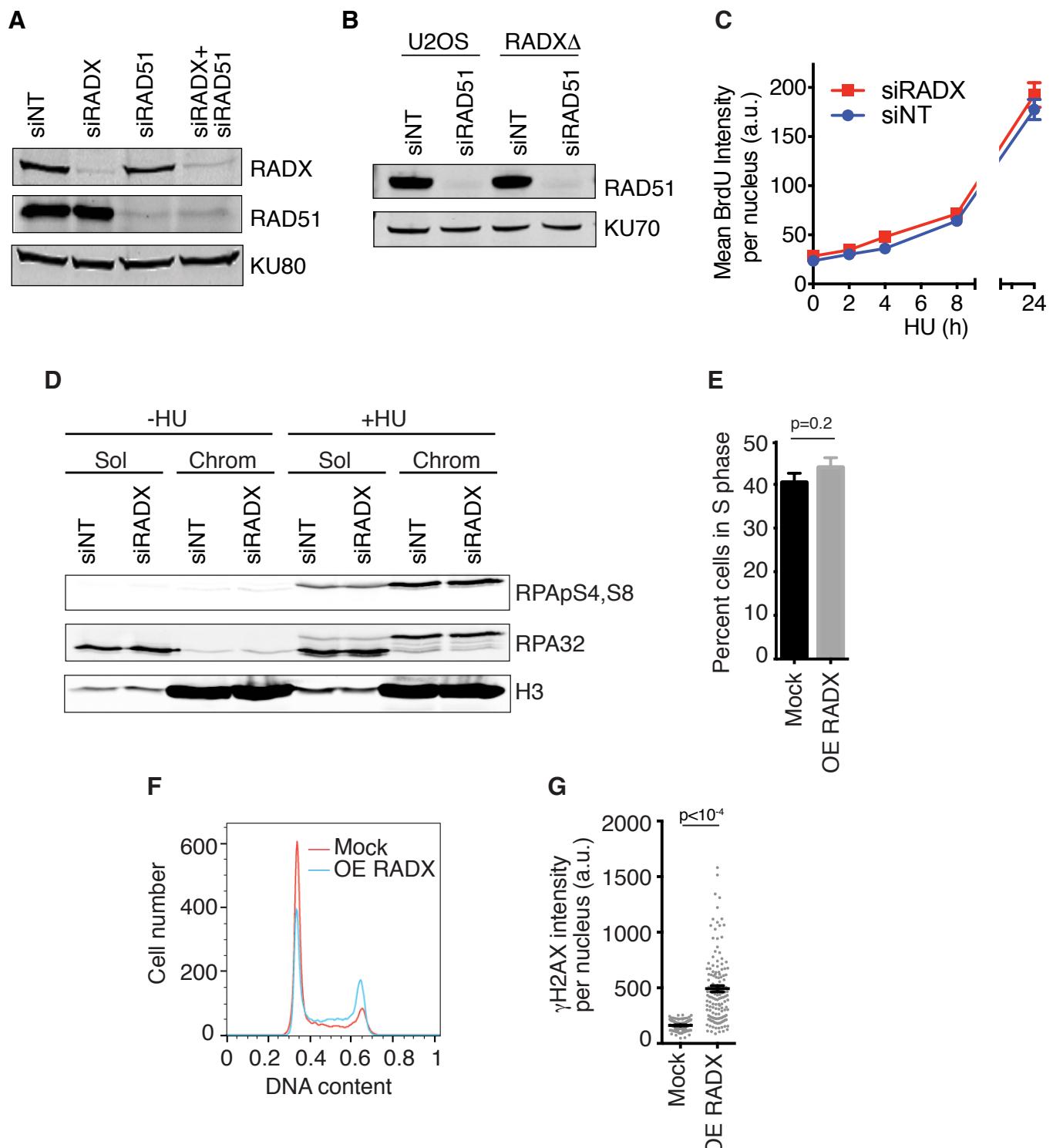


Figure S5 (related to Figures 5-7)

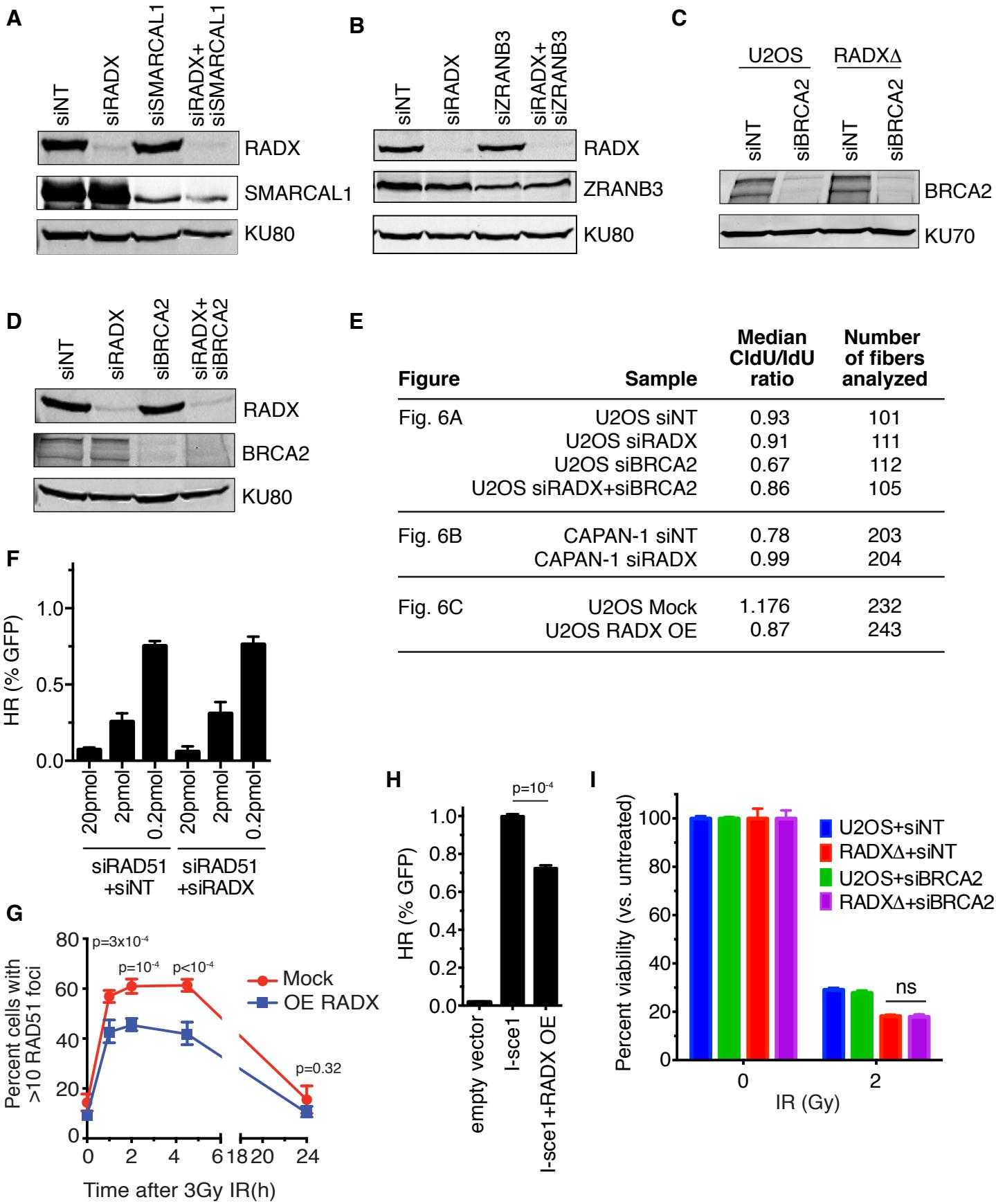


Figure S6 (related to Figure 7)

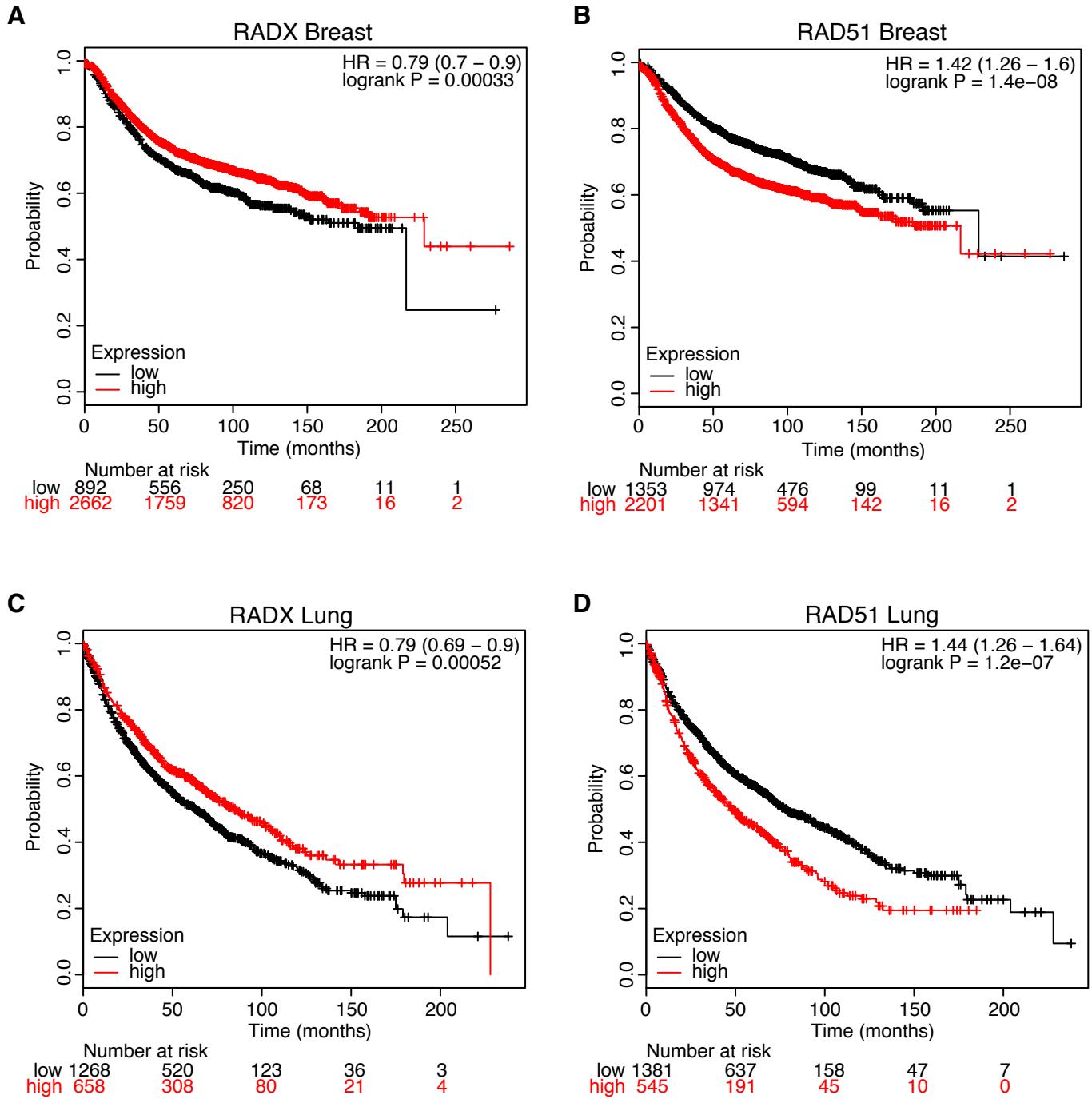
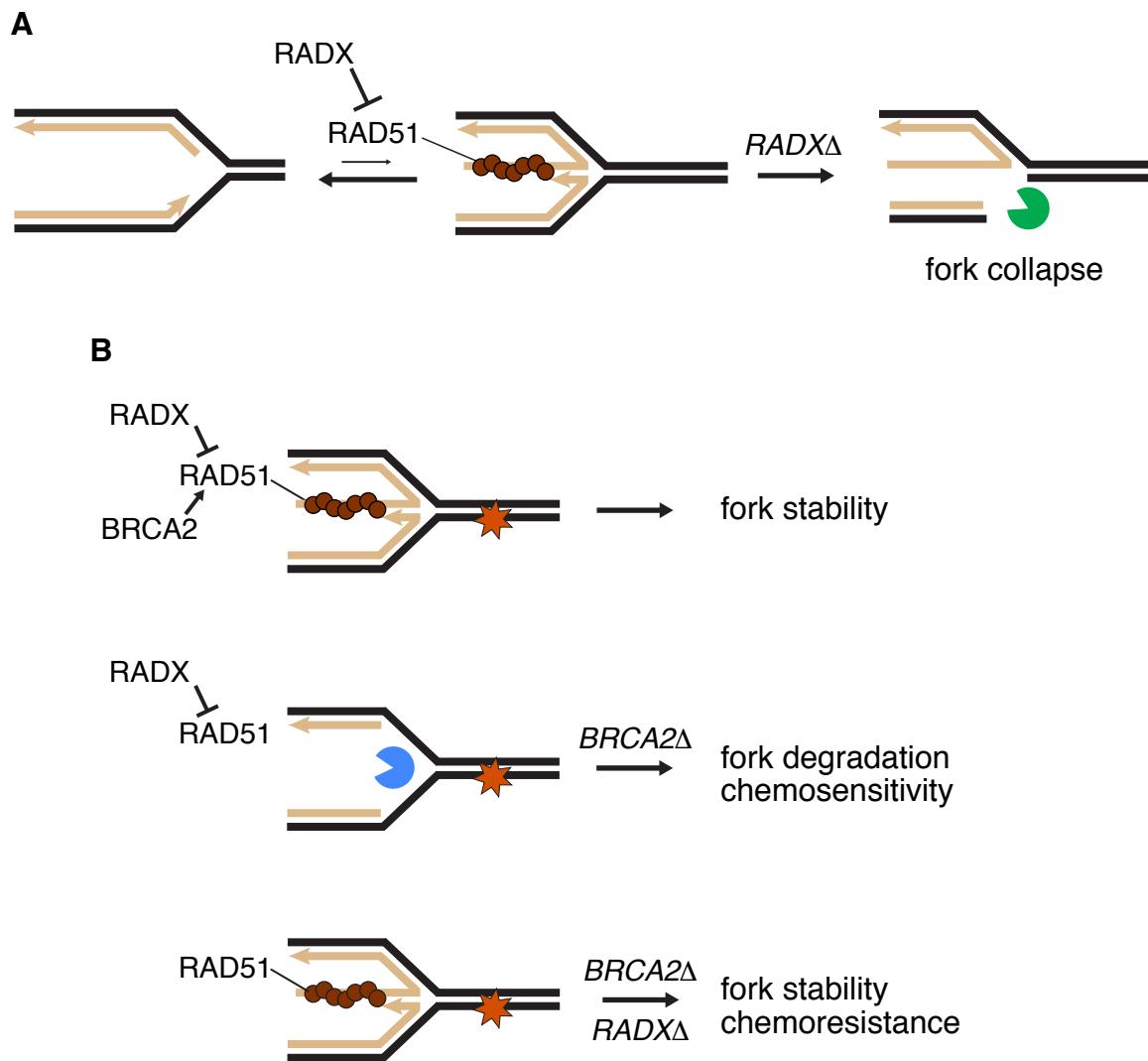


Figure S7 (related to Figures 1-7)



SUPPLEMENTAL ITEM

Table S2. List of oligonucleotides used in this study (Related to STAR METHODS: Key Resources Table)

REAGENT or RESOURCE	SOURCE	IDENTIFIER
All stars negative control siRNA (siNT)	Qiagen	Cat#SI03650318
siRADX#1: GAACACAACUUUAGCGAUA	ON-TARGETplus Dharmacon	Cat#J-014634-17
siRADX#2: CUUCAGAAAUAGAGCGCAC	ON-TARGETplus Dharmacon	Cat#J-014634-19
siRADX#3: GCUUGAACUCUCUCGUUAUA	ON-TARGETplus Dharmacon	Cat#J-014634-20
siRADX#4: CAUAGAGGCCAGCCGUUAUA	ON-TARGETplus Dharmacon	Cat#J-014634-21
siMUS81: GGGUUAUACCUGGUGGAAGA	siGENOME Dharmacon	Cat#D-016143-04
siRAD51#1: AAGGGAATTAGTGAAGCCAAA	Qiagen Flexitube	Cat#SI02663682
siRAD51#2: CAGGTGGTAGCTCAAGTGGAT	Qiagen Flexitube	Cat#SI03072272
siBRCA2: CAGGACACAAATTACAACACTAAA	Qiagen Flexitube	Cat#SI02653434
siSMARCAL1: GCUUUGACCUUCUUAGCAA	ON-TARGETplus Dharmacon	Cat#J-013058-06
siZRANB3: CAAGAGAUAUCAUCGAUUAtt	Ambion Silencer Select	Cat#4392420
siBLM#1: GAGCACACUUGUAAAUA	siGENOME Dharmacon	Cat#D-007287-01
siBLM#2: GAGAACACUACUUCAAUAA	siGENOME Dharmacon	Cat#D-007287-03
siBLM#3: CAGGAUGGCUGUCAGGUUA	siGENOME Dharmacon	Cat#D-007287-04
siBLM#4: CUAAAUCUGUGGAGGGUUA	siGENOME Dharmacon	Cat#D-007287-05
Guide RNA RADX#1: 3'-CACCGAATCAAAACTGCGATACTA-5'	This paper	NA
Guide RNA RADX#2: 3'-CACCGTTACCATTACATGTTAAC-5'	This paper	NA
RADXmRNA(Mouse) Forward primer 5'-TTGGAGCACCCGAAAGGGATCAGG	This paper	NA
RADXmRNA(Mouse) Reverse primer: 5' ATCTAGGGACTCCCCACAGTGGACC	This paper	NA